Table 1

Saprolegnia diclina (ATCC 56851) Desaturase Expression in Baker's Yeast

CLONE	TYPE OF ENZYME ACTIVITY	% CONVERSION OF SUBSTRATE	
pRSP1	Δ9	0	(18:0 to 18:1n-9)*
(*S. diclina* Δ6 desaturase)	Δ12	0	(18:1 to 18:2n-6)
	Δ15	0	(18:2n-6 to 18:3n-3)
	Δ6	28	(18:2n-6 to 18:3n-6)
	Δ6	37	(18:3n-3 to 18:4n-3)
	Δ5	0	(20:3n-6 to 20:4n-6)
pRSP3	Δ9	0	(18:0 to 18:1n-9)
(*S. diclina* Δ5 desaturase)	Δ12	0	(18:1 to 18:2n-6)
	Δ15	0	(18:2n-6 to 18:3n-3)
	Δ6	0	(18:2n-6 to 18:3n-6)
	Δ6	0	(18:3n-3 to 18:4n-3)
	Δ5	27	(20:3n-6 to 20:4n-6)

***above endogenous Δ9 activity**

Table 2

Fatty Acid as a Percentage of Total Lipid Extracted from Yeast

Clone	18:1* Present	18:2 (n-3) Produced	18:2 (n-6) Incorporated	18:3 (n-3) Produced	18:3 (n-6) Produced	18:3 (n-3) Incorporated	18:4 (n-3) Produced	20:3(n-6) Incorporated	20:4 (n-6) Produced
pYX242 (control)	15.07	0	11.14	0	0	11.35	0	11.55	0
pRSP1 (Δ6)	14.41	0	6.31	0	2.44	7.95	4.63	13.70	0
pRSP3 (Δ5)	15.34	0.08	10.72	0	0	10.43	0	20.69	7.74

50 μM substrate added
*18:1 is an endogenous fatty acid in yeast

Key:
18:1 = Oleic acid
18:2 (n-6) = Linoleic acid
18:3 (n-3) = α-Linolenic acid
18:3 (n-6) = γ-Linolenic acid
18:4 (n-3) = Stearidonic acid
20:3 (n-6) = Dihomo-γ-linolenic acid
20:4 (n-6) = Arachidonic acid

Table 3

Fatty Acid as a Percentage of Total Lipid Extracted from Yeast

Clone	Plasmid in yeast (enzyme)	18:2 (n-6) Incorporated	18:3 (n-6) Produced	20:3 (n-6) Produced	% Conversion	18:3 (n-3) Incorporated	18:4 (n-3) Produced	20:4 (n-3) Produced	% Conversion
Control	pYX242 + pYES2	6.46	0	0	0	13.26	0	0	0
pRSP5	pRSP1 (Δ6) + pRAE73-A3 (human elongase)	4.62	1.95	0.8	37.3	7.00	2.47	1.20	34.39
pRSP8	pRSP1 (Δ6) + pRPB2 (M. alpina elongase)	4.08	2.31	0.63	41	5.93	2.01	0.85	32.53

50 μM substrate added

Key:
18:2 (n-6) = Linoleic acid
18:3 (n-3) =α-Linolenic acid
18:3 (n-6) = γ-Linolenic acid
18:4 (n-3) = Stearidonic acid
20:3 (n-6) = Dihomo-γ-linolenic acid
20:4 (n-3) = Eicosatetraenoic acid

$$\% \text{ Conversion} = \frac{[\% \text{ Product 1} + \% \text{ Product 2}]}{[\% \text{ substrate} + \% \text{ Product 1} + \% \text{ Product 2}]}$$

Table 4

Fatty Acid as a Percentage of Total Lipid Extracted from Yeast

Clone	Plasmid in yeast (enzyme)	18:3 (n-6) Incorporated	20:3 (n-6) Produced	20:4 (n-6) Produced	% Conversion	18:4 (n-3) Incorporated	20:4 (n-3) Produced	20:5 (n-3) Produced	% Conversion
Control	pYX242 + pYES2	8.17	0	0	0	5.61	0	0	0
pRSP7	pRSP3 (Δ5) + pRAE73-A3 (human elongase)	6.25	2.30	1.63	38.6	4.12	1.98	1.56	46.2
pRSP10	pRSP3 (Δ5) + pRPB2 (M. alpina elongase)	7.00	2.07	1.35	32.82	4.66	1.50	1.61	40.02

50 μM substrate added

Key:
18:3 (n-6) = γ-Linolenic acid
18:4 (n-3) = Stearidonic Acid
20:3 (n-6) = Dihomo-γ-linolenic acid
20:4 (n-6) = Arachidonic Acid
20:4 (n-3) = Eicosatetraenoic acid
20:5 (n-3) = Eicosapentanoic Acid

$$\% \text{ Conversion} = \frac{[\% \text{ Product 1} + \% \text{ Product 2}]}{[\% \text{ substrate} + \% \text{ Product 1} + \% \text{ Product 2}]}$$

Table 5

Thraustochytrium aureum (ATCC 34304) Desaturase Expression in Baker's Yeast

CLONE	TYPE OF ENZYME ACTIVITY	% CONVERSION OF SUBSTRATE	
PRTA4	Δ9	0	(18:0 to 18:1n-9)*
(*T. aureum* Δ5 desaturase)	Δ12	0	(18:1 to 18:2n-6)
	Δ15	0	(18:2n-6 to 18:3n-3)
	Δ6	0	(18:2n-6 to 18:3n-6)
	Δ6	0	(18:3n-3 to 18:4n-3)
	Δ5	23.7	(20:3n-6 to 20:4n-6)
	Δ17	0	(20:4n-6 to 20:5n-3)
	Δ19	0	(22:4n-6 to 22:5n-3)
	Δ4	0	(22:4n-6 to 22:5n-6)
	Δ4	0	(22:5n-3 to 22:6n-3)

***above endogenous Δ9 activity**

Table 6

Fatty Acid as a Percentage of Total Lipid Extracted from Yeast

Clone	18:1* Present	18:2 Produced	18:2(n-6) Incorporated	18:3(n-6) Produced	18:3(n-6) Produced	18:3(n-3) Incorporated	18:4(n-3) Produced	20:3(n-6) Incorporated	20:4(n-6) Produced
PYX242 (control)	32.13	0	8.68	0	0	54.42	0	4.3	0
PRTA4 (Δ5)	29.67	0	11.18	0	0	9.93	0	21.94	6.84

50 μM substrate added
*18:1 is an endogenous fatty acid in yeast

Key:
18:1 =Oleic acid
18:2(n-6) =Linoleic acid
18:3(n-3) =α-Linolenic acid
18:3(n-6) =γ-Linolenic acid
18:4(n-3) =Stearidonic acid
20:3(n-6) =Dihomo-γ-linolenic acid
20:4(n-6) =Arachidonic acid

Table 7

Fatty Acid as a Percentage of Total Lipid Extracted from Yeast

Clone	20:3 Incorporated	20:4 Produced	22:4 Produced	Conversion to products
PYX242/ PYES2 (control)	41.98	0	0	0
PRTA4(Δ5)/ PRAE73-A3 (human elongase)	15.59	4.2	6.28	16.7

100 μM substrate added
*18:1 is an endogenous fatty acid in yeast

Key:
γ-18:3 =γ-Linolenic acid
20:3 =Dihomo-γ-linolenic acid
20:4 =Arachidonic acid
22:4 =Adrenic acid

Table 8 Fatty acid profiles of yeast containing pRAT-2c, pYX242, pRAT-2c/pRAE-73-A3, or pYX242/pYES2, grown in the presence of various fatty acids.

plasmid	pRAT-2c	pYX242	pRAT-2c pRAE-73	pYX242 pYES2	pRAT-2c pRAE-73	pYX242 pYES2	pRAT-2c pRAE-73	pYX242 pYES2
100 μM	DGLA	DGLA	LA	LA	ALA	ALA	GLA	GLA
			g/100 g Fatty Acid					
C18:2n-6			13.98	18.49				
C18:3n-6							15.97	15.1
C18:3n-3					10.27	14.14		
C18:4n-3								
C20:2n-6			0.59	0.27				
C20:3n-6	33.37	32.4					1.25	
C20:4n-6	22.98						0.83	
C20:3n-3					1.58	0.25		
C20:4n-3								
		% conversion = [product/(substrate + product)] x 100						
elongase			4.0%	1.4%	13.3%	1.7%	11.5%	
Δ5	40.8%						39.9%	

Table 9 Fatty acid profiles of yeast containing pRAT-1a, pYX242, pRAT-1a/pRAE-73-A3, or pYX242/pYES2, grown in the presence of various fatty acids.

Plasmid 100 µM	pRAT-1a LA	pYX242 LA	pRAT-1a ALA	pYX242 ALA	pRAT-1a EDA	pYX242 EDA	pRAT-1a DGLA	pYX242 DGLA	pRAT-1a pRAE-73 LA	pYX242 pYES2 LA	pRAT-1a pRAE-73 ALA	pYX242 pYES2 ALA
					g/100 g Fatty Acid							
C18:2n-6	18.44	20.91							11.35	18.49		
C18:3n-6	2.62								3.49			
C18:3n-3			11.59	12.38							4.61	14.14
C18:4n-3			3.37								3.69	
C20:2n-6					55.6	55.55			0.21	0.27		
C20:3n-6					2.35		66.37	32.4	1.1			
C20:4n-6							0.78					
C20:3n-3											0.65	0.25
C20:4n-3											4.32	
					% conversion = [product/(substrate + product)] x 100							
Δ6	12.4%		22.5%									
Δ5							1.2%					
Δ8					4.0%							